Secondary Mould Metabolites. Part 36.[1] Isolation and Structure Elucidation of Sulcatines C–E, Novel Norsesquiterpenes from *Laurilia sulcata*, and 7-*epi*-Sulcatine D

Alberto Arnone,[a] Gianluca Nasini,[a] Orso Vajna de Pava[a] and Gemma Assante[b]
[a] *Dipartimento di Chimica del Politecnico, Centro del C.N.R. per le Sostanze Organiche Naturali, Politecnico di Milano, Piazza L. da Vinci 32, I 20133 Milano, Italy*
[b] *Istituto di Patologia Vegetale, Facoltà di Agraria, Università di Milano, Via Celoria 2, I 20133 Milano, Italy*

Three novel norsesquiterpenes, sulcatines C–E (**5**, **6** and **11**), have been isolated from still liquid cultures of *Laurilia sulcata*. Their structures were elucidated by ^{1}H and ^{13}C NMR spectroscopic studies, including ^{1}H–^{13}C heteronuclear correlation spectra (HETCOR and COLOC), and the relative configurations were established through a series of NOE difference spectra and the magnitude of the ^{1}H–^{1}H coupling constants. The absolute configuration of these metabolites was deduced by the application of the exciton chirality method to the pivalate dibenzoate of sulcatine D, compound **9**. Sulcatines C and E (**5** and **11**) were shown to exist in solution as an equilibrating mixture of ring-closed hemiketal (**5a** and **11b**) and ring-opened keto (**5c** and **11a**) forms. By reduction with $NaBH_4$ sulcatine C **5** afforded a separable mixture of sulcatine D **6** and its C-7 epimer **10**.

Strains of Basidiomycetes have been shown to produce biologically active sesquiterpenes, the majority of which have the protoilludyl cation as a biosynthetic intermediate; among these are *Clitocybe illudens* (= *Omphalotus olearius*) and *Fomes annosus*, which produce illudins[2] and fomannosin,[3] *Armillaria mellea*, whose metabolites have been extensively examined by several groups,[4] and recently *Laurilia sulcata*.

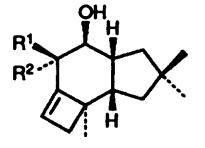
The latter fungus has been reported to produce sulcatine A **1**, a Δ^5-norprotoilludene-7,8-diol, when grown in agar cultures[5] and sulcatine B **2**, armillol **3** and 5-*epi*-armillol **4** when grown in still liquid cultures.[1]

In this paper we describe the isolation of three additional metabolites, named sulcatines C–E, compounds **5**, **6** and **11**, produced by liquid cultures of *Laurilia sulcata*, and 7-*epi*-sulcatine D **10** obtained, together with sulcatine D **6**, by $NaBH_4$ reduction of sulcatine C **5**. The structure and the stereochemistry of these compounds followed from ^{1}H and ^{13}C NMR studies, NOE difference experiments, and CD data. Complete assignment of all the resonances contained in the ^{1}H and ^{13}C NMR spectra of compounds **5**, **6**, **10**, **11** and **12** (Tables 1 and 2), except for those belonging to the minor tautomeric forms of sulcatines C and E (**5c** and **11b**), was achieved by carrying out two-dimensional correlation spectroscopy optimized for the observation of one-bond ^{1}H–^{13}C couplings of ~140 Hz (HETCOR) and two- and three-bond ^{1}H–^{13}C couplings of ~6 Hz (COLOC).

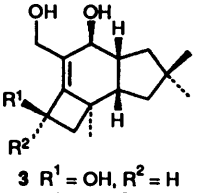
Sulcatine C **5** was isolated as crystals, m.p. 105–108 °C; $[\alpha]_D$ +58.7 10^{-1} deg cm^2 g^{-1} (*c* 0.1, $CHCl_3$), that analysed for $C_{14}H_{22}O_3$; chemical ionisation mass spectroscopy gave a distinct peak at *m/z* 239 (MH^+) and a fragment at *m/z* 221 [(MH^+) − 18, base peak] due to the loss of water.

Inspection of the ^{1}H and ^{13}C NMR spectra of sulcatine C revealed that this compound exists in solution as a solvent-dependent mixture of the hemiketal and keto forms, **5a** and **5c**, whose ratio is ~97:3 and 90:10 in [$^2H_6$]acetone and $CDCl_3$, the intermediate hemiacetal form **5b** (see Scheme 1) being absent in both solvents.

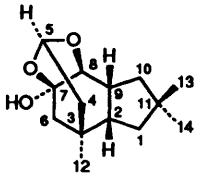
The broad-band ^{1}H-decoupled ^{13}C NMR spectrum of the major form **5a** in [$^2H_6$]acetone exhibited 14 signals, which were assigned using DEPT experiments to three methyl, four methylene, four methine, and three quaternary carbon atoms. A detailed analysis of the corresponding ^{1}H NMR spectrum revealed the presence of the –C(1)H_2–C(2)H–C(9)H–C(10)H_2– grouping which must be part, together with the quaternary C-11 carbon, of a cyclopentane ring since the protons of both the tertiary methyl groups resonating at δ_H 1.08 and 0.97 presented in the COLOC spectrum depicted in Fig. 1, with cross-peaks with C-1, C-10 and C-11. Additional cross-peaks observed between the remaining tertiary methyl group at δ_H 0.82 and C-2, C-3, C-4 and C-6 completed partial structure **A**.



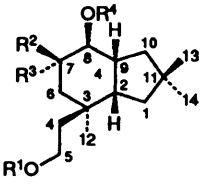
1 R^1 = H, R^2 = OH
2 R^1 = OH, R^2 = CH_2OH



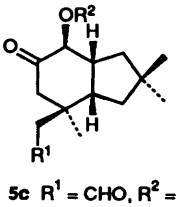
3 R^1 = OH, R^2 = H
4 R^1 = H, R^2 = OH



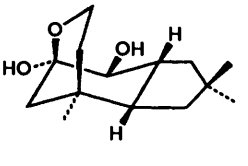
5a



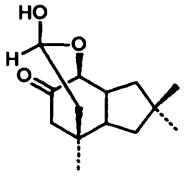
6 R^1 = R^2 = R^4 = H, R^3 = OH
7 R^1 = R^4 = Ac, R^2 = H, R^3 = OAc
8 R^1 = COBu^t, R^2 = R^4 = H, R^3 = OH
9 R^1 = COBu^t, R^2 = H, R^3 = OBz, R^4 = Bz
10 R^1 = R^3 = R^4 = H, R^2 = OH



5c R^1 = CHO, R^2 = H
11a R^1 = CH_2OH, R^2 = H
12 R^1 = CH_2OAc, R^2 = Ac



11b



5b

Table 1 ^{1}H NMR data for compounds **5a**, **6**, **10**, **11a**, **11b** and **12** in [$^2H_6$]acetone

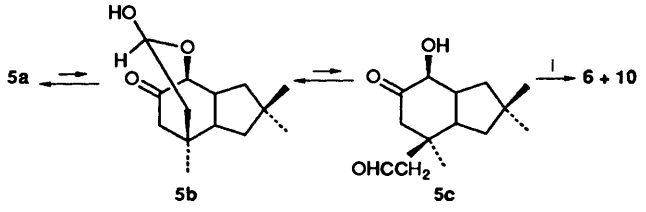
	δ_H						J/Hz						
Proton	**5a**[a]	**6**	**10**	**11a**	**11b**	**12**	J(H,H)[b]	**5a**	**6**[c]	**10**	**11a**	**11b**	**12**
1α	1.35	1.47 (1.38)[d]	1.46	1.84	1.4–1.8	1.99	1α,1β	13.3	12.2	12.3	12.3	*f*	12.5
1β	1.52	1.35 (1.27)	1.36	1.60	1.4–1.8	1.68	1α,2	9.2	13.4	13.6	12.7	12.5	12.9
2	2.17	1.98 (1.84)	1.92	2.43	1.96	2.37	1β,2	8.6	7.0	7.1	6.5	7.2	6.7
4a	1.54	1.77 (1.65)	2.27	1.48	1.4–1.8	1.61	1β,9	~0	0.7	1.0	1.3	*f*	1.1
4b	1.47	1.58 (1.44)	1.42	1.48	1.4–1.8	1.61	2,6β	2.2	1.5	1.7	1.5	1.7	1.7
5a	5.27	3.63 (3.47)	3.76	3.63	3.86	4.18	2,9	10.6	6.2	6.0	6.1	7.2	6.2
5b		3.63 (3.47)	3.63	3.63	3.84	4.08	4a,4b	14.0	14.0	14.2	*f*	*f*	*f*
6α	1.90	1.34 (1.23)	1.46	2.65	1.4–1.8	2.79	5a,5b		*f*	10.5	10.5[e]	11.7[e]	11.0
6β	1.57	1.56 (1.43)	1.90	2.07	1.4–1.8	2.09	6α,6β	13.0	13.3	15.1	13.0	*f*	13.4
7		3.48 (3.33)	3.87				6α,7		12.0	3.0			
8	3.63	3.16 (3.01)	3.35	4.04	3.16	5.10	6β,7		4.5	3.5			
9	2.85	1.99 (1.86)	2.19	2.18	2.28	2.49	7,8		9.5	3.4			
10α	1.19	1.82 (1.70)	1.81	1.87	1.4–1.8	1.56	8,9	2.6	10.5	10.9	9.8	10.3	11.4
10β	1.40	1.52 (1.44)	1.52	1.69	1.4–1.8	1.71	9,10α	13.7	1.0	1.2	1.2	*f*	1.3
12	0.82	0.87 (0.79)	0.83	1.03	0.93	1.09	9,10β	7.4	7.3	7.2	6.8	*f*	6.8
13	0.97	1.01 (0.98)	1.02	1.09	1.00	1.10	10α,10β	12.2	13.6	13.6	13.6	*f*	13.8
14	1.08	1.12 (1.10)	1.12	1.22	1.11	1.20							
5-OR		3.30 (4.25)	3.35	3.49		1.98							
7-OH	5.68	3.30 (4.33)	3.35		4.48								
8-OR		3.30 (4.30)	3.35	3.78	3.29	2.08							

[a] Selected data for compound **5c** in $CDCl_3$: 9.81 (dd, *J* 3.0 and 2.0, 5-H), 4.04 (dd, *J* 9.5 and 1.3, 8-H), 2.64 (br d, *J* 13.8, 6-H^a), 2.38 (dd, *J* 13.8 and 1.5, 6-H$^\beta$), 2.37 (br dd, *J* 15.5 and 2.0, 4-H^a), 2.31 (br dd, *J* 15.5 and 3.0, 4-H^b) and 2.20 (m, 9-H). [b] Additional coupling constants: $J_{1\beta,10\beta}$ 2.3, $J_{4a,5}$ 2.3, $J_{4b,5}$ 1.7, and $J_{4b,6\alpha}$ 1.6 for compound **5a**; $J_{4a,5a} + J_{4a,5b}$ 15.5, $J_{4b,5a} + J_{4b,5b}$ 15.0, $J_{5,5\text{-OH}}$ 5.0, $J_{7,7\text{-OH}}$ 4.5, and $J_{8,8\text{-OH}}$ 4.5 (in [$^2H_6$]DMSO) for compound **6**; $J_{4a,5a}$ 8.4, $J_{4a,5b}$ 5.7, $J_{4a,12}$ 0.9, $J_{4b,5a}$ 5.0, $J_{4b,5b}$ 5.3, and $J_{4b,6\alpha}$ 1.3 for compound **10**; $J_{4a,5a} + J_{4b,5a}$ 14.5 and $J_{4a,5b} + J_{4b,5b}$ 14.5 (in $CDCl_3$), $J_{6\alpha,8}$ 1.3, $J_{5,5\text{-OH}}$ 5.0, and $J_{8,8\text{-OH}}$ 4.5 for compound **11a**; $J_{4a,5a} + J_{4b,5a}$ 14.5 and $J_{4a,5b} + J_{4b,5b}$ 12.0 (in $CDCl_3$) and $J_{8,8\text{-OH}}$ 6.5 for compound **11b**; and $J_{6a,8}$ 1.3 Hz for compound **12**. [c] In [$^2H_6$]acetone + [$^2H_6$]benzene 1:5. [d] Values in parentheses are chemical shifts in [$^2H_6$]DMSO. [e] In $CDCl_3$. [f] Not assigned.

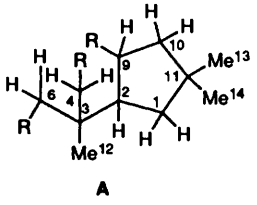
Table 2 ^{13}C NMR data for compounds **5a**, **10**, **11a**, **11b** and **12** in [$^2H_6$]acetone

	5a		**6**		**10**		**11a**		**11b**		**12**	
Carbon	δ_C	^1J(C,H)	δ_C	^1J(C,H)	δ_C	^1J(C,H)	δ_C	^1J(C,H)	δ_C	^1J(C,H)	δ_C[a]	^1J(C,H)
1	43.99	127	42.62	127	42.14	126	42.26	127	42.98[b]	126	42.14	127
2	46.09	131	50.90	126	51.79	125	49.33	126	51.96	126	49.19	126
3	35.80		35.32		33.29		40.36		33.24		40.13	
4	47.72	126.5	43.96	125	45.32	125	43.75	125	37.42[b]	128	39.49	125
5	100.53	169.5	58.78	139	59.01	139.5	58.19	140	61.24	143.5	61.03	147
6	39.34	127	40.09	125	36.12	124	47.24	129	40.60[b]	125	47.86	130
7	102.38		71.98	139	70.66	143	211.34		96.59		204.07	
8	79.64	151	77.49	138	72.19	137	76.65	140	79.22	139	78.41	144
9	42.86	130	45.53	131	41.44	130	49.95	133.5	46.19	129	45.86	133
10	43.41	128	44.32	127	44.08	126	45.42	129	45.85[b]	*c*	45.33	129
11	39.95		37.05		36.82		37.42		37.26		37.54	
12	27.37	125	26.81	125	27.54	125	25.97	125	29.51	125	25.62	126
13	26.97	124.5	33.28	125	33.38	125	33.07	125	32.26	124.5	32.98	125
14	29.42	124.5	33.28	125	33.29	125	33.18	125	32.49	124.5	33.10	125

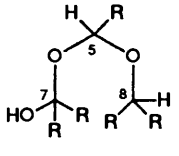
[a] The carbonyl and methyl carbon atoms of the 5- and 8-acetate groups resonate at δ 170.88, 170.52 and 20.89 ($^1J_{C,H}$ 129.5 Hz), and 20.54 ($^1J_{C,H}$ 129.5 Hz). [b] Assignments may be interchanged. [c] Not assigned.



Scheme 1 *Reagents*: i, $NaBH_4$, MeOH



A



B

The $C_3H_3O_3$ fragment shown in partial structure **B** contains acetal, hemiketal and ethereal functions as evidenced by the chemical-shift values exhibited by C-5, C-7 and C-8 (δ_C 100.53, 102.38 and 79.64) and by the presence of the tertiary hydroxy proton at δ_H 5.68 (7-OH).

The cross-peaks observed between 5-H and C-3, C-4, C-7 and C-8 defined the mode of linkage between C-4 and C-5 while the presence of a ^{1}H–^{1}H coupling between 8- and 9-H and not between 8-H and 6-H_2 allowed us to connect C-8 to C-9. Thus, to assign the gross structure to the hemiketal form of sulcatine C we had only to link C-7 to C-6 and C-8.

The NOE difference experiments reported in Table 3 established the relative configuration at C-2, -3, -5, -7, -8 and -9 as

Table 3 Selected connectivities established by NOE difference experiments for compounds **5a**, **6**, **10**, **11a** and **12**[a]

Proton irradiated	Proton affected (%) in compound				
	5a[b]	**6**	**10**	**11a**	**12**[c]
7		4a(3.5), 4b(3.5), 6β(2.5), 9 (3.5)	6a(3.5), 6β(3.5), 8(2)		
8	9(3), 10α(1), 10β(1)	1α(2.5), 6α(3), 10α(3.5), 14 (0.5)	1α(2.5), 6α(1.5), 7(3.5), 10α(2), 14(1)	1α(3), 6α(3), 10α(3), 14(1)	1α(3.5), 6α(2.5), 10α(3.5), 14 (1)
12	1α(5), 2(3), 4a(4), 4b(2.5), 6α(1.5), 6β(3.5)	1α(2), 1β(3), 2(4.5), 4a(1), 4b(3), 5a and 5b(4), 6α(3.5), 6β(2.5)	1α(1), 1β(4.5), 2(2), 4b(3.5), 5a(4.5), 5b(1.5), 6α(2.5), 6β(2.5)	1α(1.5), 1β(5), 2(2), 4a and 4b(1.5), 5a and 5b(3), 6α(3), 6β(2)	1α(1), 1β(8), 2(5), 4a and 4b(1.5), 5a(3.5), 5b(3.5), 6α(4), 6β(2.5), 10β(5)
13	1β(3.5), 2(2.5), 9(6), 10β(5)	1β(3), 2(6), 10β(4)	1β(4), 2(6.5), 10β(4.5)	1β(5), 2(2.5), 10β(4.5), 14(2)	
14	1α(2.5), 1β(1), 10β(2)	1α(2.5), 8(7.5), 10α(4.5)	1α(4), 8(4.5), 10α(4.5)	1α(6), 8α(4.5), 10α(5.5), 13 (1.5)	1α(4.5), 8(6), 10α(5)

[a] NOEs obtained in [$^2H_6$]acetone for compounds **5a** and **12**, in [$^2H_6$]DMSO + D_2O for compound **6** and in [$^2H_6$]acetone + D_2O for compounds **10** and **11a**. [b] Additional NOEs: {2-H} enhanced 1α(0.5), 1β-(2), 4b(2.5), 9 (3.5), 12(0.5), 13(0.5); {5-H} enhanced 4a(1.5), 4b(1.5), 8(0.5); {6α-H} enhanced 1α(4), 6β(12), 10α(2); {7-OH)} enhanced 6α(1), 6β(1), 8(5), 10α(0.5). [c] 12- and 13-Methyl protons were irradiated together. Additional NOEs: {2-H} enhanced 1β(3), 4a and 4b(1), 5a(2), 5b(1), 10β(3), 12(0.5), 13(1); {9-H} enhanced 4a and 4b(2), 5a(1), 5b(0.5), 10α(1), 10β(3); 2- and 9-H resonate too close for the NOE to be observed.

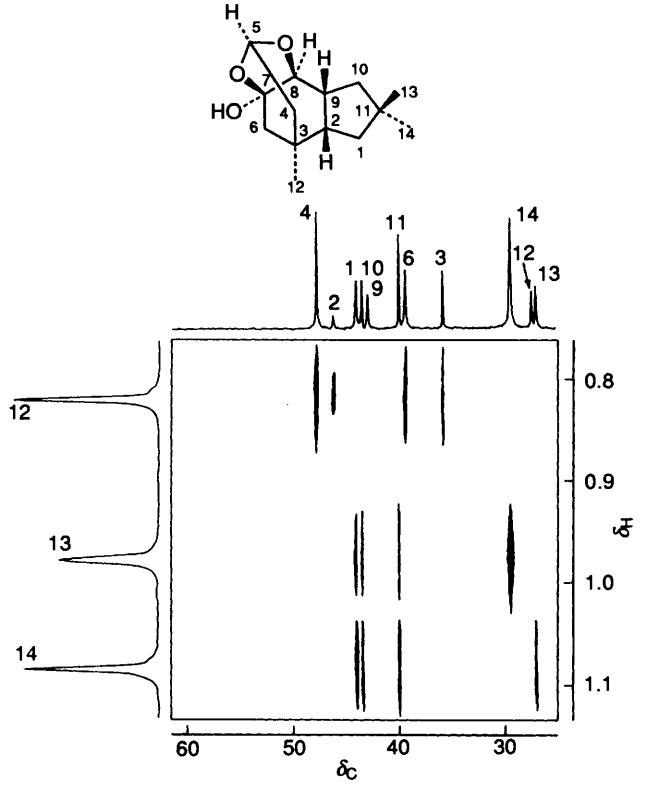


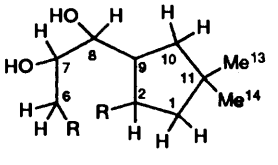
Fig. 1 Selected portion of the COLOC spectrum of sulcatine C **5a** in [$^2H_6$]acetone, optimised for the observation of long-range ^1H–^{13}C couplings of ~6 Hz

*S**,*S**,*S**,*R**,*S**,*R**, it having been assumed that C-2 has the *S**-configuration. The NOEs observed between 13-H_3, assumed as β, and 2-H (2.5%) and 9-H (6%) and between 2-H and the 4-methylene proton at δ_H 1.47 (2.5%) imply that all these protons are on the same β-side of the molecule. These results not only define the relative configuration at C-2, C-3 and C-9 as *S**,*S**,*R** but also require that the two oxygen atoms forming the acetal group are β-disposed to give structure **5a**, thus allowing the assignment of the chirality at C-7 and C-8 as *R** and *S**.

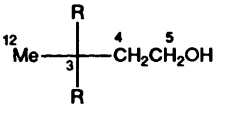
In addition to the major isomer **5a** present in solution, there is also a small amount of the keto aldehyde isomer **5c** present, as evidenced in the ^1H NMR spectrum in $CDCl_3$ by a double doublet at δ_H 9.81 due to an aldehydic proton and by the conversion of sulcatine C into a 1:1 mixture of sulcatine D **6** and its C-7 epimer **10** *via* reduction with $NaBH_4$ of the C-5 and C-7 carbonyl groups.

Sulcatine D **6** was obtained as crystals, m.p. 116–118 °C; $[\alpha]_D$ −29.4 10^{-1} deg cm^2 g^{-1} (*c* 0.2, $CHCl_3$); elemental analysis and CI mass spectroscopy indicated the formula $C_{14}H_{26}O_3$.

The broad-band ^1H-decoupled ^{13}C NMR spectrum, together with DEPT experiments, revealed the presence of 14 carbons due to three methyl, five methylene, four methine and two quaternary carbon atoms. The ^1H NMR spectrum in $(CD_3)_2SO$ ([$^2H_6$]DMSO) exhibited three tertiary methyl groups, signals attributable to a $-C(4)H_2-C(5)H_2OH$ grouping, and two additional exchangeable hydroxy resonances vicinally coupled to 7- and 8-H). Decoupling experiments, as corroborated by the values of the vicinal ^1H–^1H coupling constants, and the cross-peaks observed in the COLOC spectrum depicted in Fig. 2 between both 13- and 14-H_3 and C-1, -10 and -11 and between 12-H_3 and C-2, -3, -4 and -6, indicated the presence of the partial structures **A′** and **B′** and the mode of linkage of C-3 to C-2 and C-6 to give the gross structure of sulcatine D **6**.



A′



B′

The relative configuration of sulcatine D **6** was established as 2*S**,3*S**,7*S**,8*S**,9*R**, it having been assumed that C-8 has the *S**-configuration. The NOEs observed between 14-H_3, assumed as α, and 8-H (7.5%) and between 13-H_3 and 2-H (6%) require that 8- and 2-H are α-and β-disposed; the NOE observed between 7- and 9-H (3.5%) and the values of 9.5 and 10.5 Hz exhibited by 7-H and 8-H^α and 8-H^α and 9-H, indicating *trans*-relationships, imply that 7- and 9-H are β-disposed; and the NOE observed between 7-H^β and 4-H_2 (3.5%) establishes their *cis* disposition. The above findings together with the values observed for the coupling constants of the cyclopentane protons indicate that sulcatine D **6** adopts in solution the preferred conformation shown in Fig. 2 in which the cyclohexane ring assumes a chair-like geometry and the cyclopentane ring

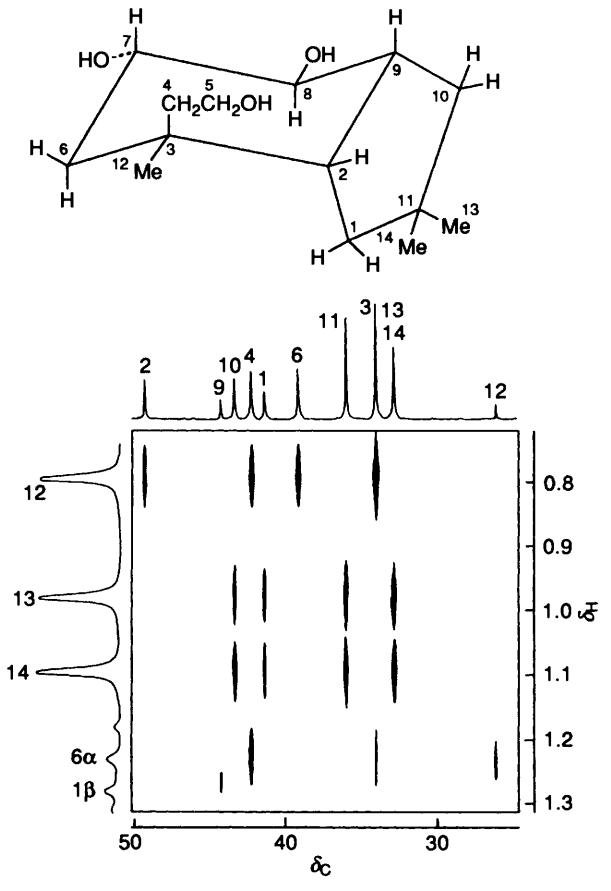


Fig. 2 Preferred conformation and selected portion of the COLOC spectrum of sulcatine D **6** *in* [$^2H_6$]*DMSO* + D_2O, optimised for the observation of long-range ^1H–^{13}C couplings of ~6 Hz

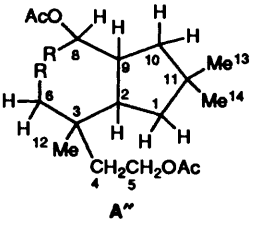
assumes an envelope-like geometry with C-1, -9, -10 and -11 nearly coplanar.

The chirality of C-7 and C-8, and thus the absolute configuration of sulcatine D **6** and also of sulcatine C **5** because of the above described interrelationship between these compounds, was determined as *S*,*S* by application of the dibenzoate chirality method[6] to the 5-pivalate 7,8-dibenzoate derivative **9**. In fact, the CD spectrum of this compound showed a positive Cotton effect, analogous to that observed for sulcatine A **1**[5] (see Experimental section), indicating the right-handedness of the orientation of the two benzoate groups.

7-*epi*-Sulcatine D **10** exhibited the same molecular weight as sulcatine D **6** and had similar ^1H and ^{13}C NMR spectra, the only differences being due to the opposite chirality at C-7. Specifically, the values of 3.0, 3.5 and 3.4 Hz observed between 7-H and 6-H$^\alpha$, 6-H$^\beta$ and 8-H$^\alpha$, respectively, together with the NOE between 6- and 8-H$^\alpha$ (1.5%) and the *trans* diaxial coupling of 10.9 Hz between 8-H$^\alpha$ and 9-H$^\beta$ indicate that the cyclohexane ring of the 7-*epi*-sulcatine D **10** assumes a chair-like conformation in which 7-H is α-equatorially oriented.

Sulcatine E **11** was isolated as a solid, m.p. 51–53 °C; $[\alpha]_D$ −23.7 10^{-1} deg cm^2 g^{-1} (*c* 0.15, $CHCl_3$); the molecular formula was established to be $C_{14}H_{24}O_3$ by elemental analysis and CI mass spectra (MH^+, 241). However, the ^1H and ^{13}C NMR spectra showed signals attributable to an equilibrating mixture of two structural isomers **11a** and **11b** since their ratio was found to be solvent dependent (4:1 in [$^2H_6$]acetone and 1:1 in $CDCl_3$).

Sulcatine E was nearly quantitatively converted into a diacetate (M^+, 324; $C_{18}H_{28}O_5$) to which structure **12** was assigned on the basis of evidence analogous to that observed for sulcatine D **6**. Analysis of the ^1H and ^{13}C NMR spectra and the cross-peaks observed in a COLOC experiment between 12-H_3 and C-2, -3, -4 and -6 and those between both 13- and 14-H_3 and C-1, -10 and -11 revealed the presence of partial structure **A″** in which the two OAc groups are located at C-5 and -8 because of

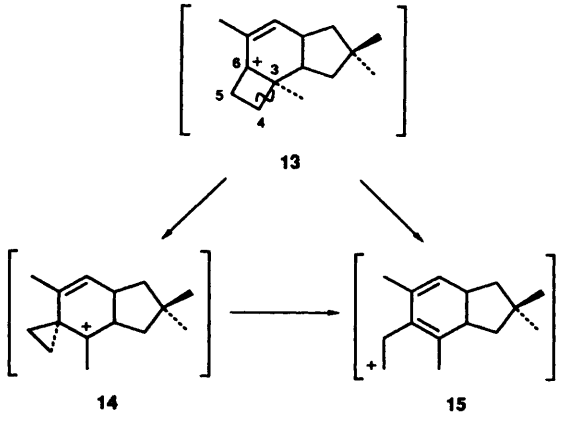


A″

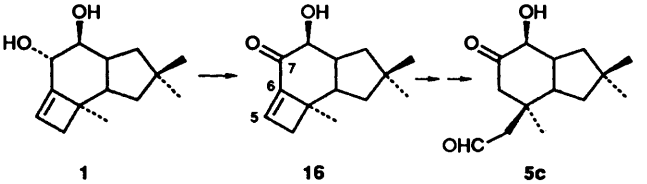
the chemical-shift values of these carbons. The remaining C-7 ketonic carbonyl carbon resonating at δ_C 204.07 must then be connected to C-6 and -8. The 2*S**,3*S**,8*S**,9*R** relative configuration for the diacetate **12** followed from the NOEs observed between 2-H and 13-H_3 (1%), assumed as β, 14-H_3 (α) and 8-H$^\alpha$ (6%), and 9-H$^\beta$ and 4-H_2 (β) (2%).

The above results and the presence in the ^{13}C NMR spectrum of sulcatine E in [$^2H_6$]acetone of two resonances for C-7 at δ_C 211.34 and 96.59 in a 4:1 ratio, characteristic of carbon atoms of ketonic and hemiketal functions, clearly indicate that this metabolite exists in solution as an equilibrating mixture of the 7-oxo and the hemiketal forms **11a** and **11b**, the chirality at C-7 in the latter being necessarily *R**.

The structures of sulcatines C–E **5**, **6** and **11** are unusual among the sesquiterpenes of protoilludane origin. Normally, the cyclobutane ring of the protoilludene intermediate **13** can be opened at the C(3)–C(4) position by hydrolitic enzymes of the fungi to give the illudane or illudalane-type cations **14** and **15**.[3,7] In our case, the opening of the four-membered ring



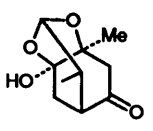
present in the co-occurring sulcatine A **1** may arise at C(5)–C(6) by a retro-aldol cleavage of a first formed C-7 keto derivative **16** (see Scheme 2), thus affording a new class of



Scheme 2

sesquiterpenoids for which we propose the name norisoilludalanes.

We have noted that the structure of sulcatine C **5a** presents some similarity with the (7*S*)-paeonimetabolite I **17**, a compound which derives from paeoniflorin by incubation with human intestinal bacteria.[8]



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All the sulcatines C–E exhibit antifungal activity. In particular, the sulcatines **5** and **11** were active in the antifungal tests performed by means of bioautography on *Cladosporium cladosporioides*, *C. cucumerinum* and *Aspergillus niger* for amounts as low as 50 μg per plate, while the sulcatines **6** and **10** are active for amounts of 100 μg per plate.

Experimental

M.p.s were determined on a Kofler apparatus and are uncorrected; IR spectra on a Perkin-Elmer 177 spectrophotometer; mass spectra on a Finnigan-MAT TSQ70 spectrometer; optical rotations on a JASCO DIP-181 polarimeter and the CD spectrum on a JASCO-500 A dichrograph. NMR spectra were recorded on a Bruker AC 250L spectrometer operating at 250.1 MHz for ^1H and 62.9 MHz for ^{13}C. Chemical shifts are in ppm (δ) from $SiMe_4$ as internal standard, and *J*-values are given in Hz. DEPT, HETCOR, and COLOC spectra were performed using the DEPT, XHCORRDC, and COLOC pulse sequences of the AC 250L software. Flash column chromatography was performed with Merck silica gel (0.04–0.063 mm), and TLC and preparative TLC (PLC) with Merck HF_{254} silica gel. Owing to the complexity of the purification procedure, we report the R_f-values in hexane–EtOAc (1:2) and CH_2Cl_2–MeOH (15:1), respectively.

Isolation and Purification of Metabolites **5**, **6** *and* **11**.—A strain of *Laurilia sulcata* (Burt) Pouzar [*Stereum sulcatum* Burt (CBS 365.49)] received from Centraal-Bureau voor Schimmel Cultures, Baarn, was maintained on MPGA (malt, peptone, glucose, agar; 20:4:20:15 g dm^{-3}) slants and sub-cultured in 40 stationary Erlenmeyer flasks (250 cm^3) containing a liquid medium MPG (50 cm^3) for 6 weeks at 24 °C; the culture filtrates which were separated from the mycelium were extracted twice with EtOAc and the extracts were dried (Na_2SO_4) and evaporated to yield a mixture (1.4 g) of sesquiterpenes. The mixture was chromatographed on a column of flash silica gel with hexane–EtOAc (2:1) as eluent, and purified further by PLC in CH_2Cl_2–MeOH (15:1) to yield the metabolites in the following order of decreasing R_f-value: sulcatine C **5** (70 mg), sulcatine E **11** (35 mg), and sulcatine D **6** (110 mg).

Sulcatine C **5**. R_f 0.5, 0.7 (Found: C, 70.5; H, 9.2. $C_{14}H_{22}O_3$ requires C, 70.55; H, 9.31%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3380 (OH), 1715 (carbonyl band), 1455, 1330 and 1320; *m/z* (CI, isobutane) 239 (MH^+), 221 [(MH^+) − 18, base peak] and 177; ^1H and ^{13}C NMR data are reported in Tables 1 and 2.

Sulcatine D **6**. R_f 0.0, 0.2 (Found: C, 69.3; H, 10.7. $C_{14}H_{26}O_3$ requires C, 69.38; H, 10.81%); *m/z* (CI, isobutane) 243 (MH^+), 225 [(MH^+) − 18], 207 [(MH^+) − 36, base peak], 189 [(MH^+) − 54], 179 and 163; ^1H and ^{13}C NMR data are reported in Tables 1 and 2.

Acetylation of Sulcatine D **6**.—Sulcatine D **6** (50 mg) was dissolved in dry pyridine (0.5 cm^3) and treated with Ac_2O (1 cm^3) overnight at 0 °C. Standard work-up followed by PLC on silica gel in hexane–EtOAc (2:1) gave the triacetate derivative **7** (40 mg) as an oil; *m/z* (CI, isobutane) 369 (MH^+), 309 [(MH^+) − 60, base peak], 267, 249 [(MH^+) − 120], 207 and 189 [(MH^+) − 180]; $\delta_H(CDCl_3)$ 5.06 (1 H, m, 8-H), 4.96 (1 H, m, 7-H), 4.22 (2 H, m, 5-H_2), 2.07 and 2.02 (9 H, s, 3 × OAc), 1.3–2.4 (10 H, m) and 1.23, 1.03 and 0.93 (9 H, s, 12-, 13- and 14-H_3).

Reaction of Sulcatine D **6** *with Pivaloyl Chloride*.—Sulcatine D **6** (100 mg), pivaloyl chloride (0.3 cm^3) and dry pyridine (1 cm^3) were left at −30 °C for 0.5 h. Standard work-up followed by PLC on silica gel in hexane–EtOAc (2:1) afforded the oily pivalate **8** (55 mg) as the main product; *m/z* (CI, isobutane) 327 (MH^+), 309 [(MH^+) − 18], 291 [(MH^+) − 36] and 207; $\delta_H(CDCl_3)$ 4.15 (2 H, m, 5-H_2), 3.63 (1 H, ddd, *J* 11.7, 9.4 and 4.5, 7-H), 3.28 (1 H, dd, *J* 9.8 and 9.4, 8-H), 1.1–2.1 (12 H, m), 1.20 (9 H, s, Bu^t) and 1.15, 1.02 and 0.92 (9 H, s, 12-, 13- and 14-H_3).

Reaction of 5-O-Pivaloyl sulcatine D **8** *with Benzoyl Chloride*.—A solution of compound **8** (10 mg) in dry pyridine (0.5 cm^3) was treated with benzoyl chloride (0.05 cm^3) at room temperature for 0.5 h; PLC in hexane–EtOAc (3:1) gave the dibenzoate **9** as an oil; *m/z* (CI, isobutane) 537 (MH^+); CD (*c* 0.13 mg cm^{-3}, 2-methylheptane) 235 and 221 nm ($\Delta\varepsilon$ +20 and −10.7); $\delta_H(CDCl_3)$ 7.2–8.1 (10 H, m, ArH), 5.50 (1 H, dd, *J* 11.0 and 10.0, 8-H), 5.28 (1 H, ddd, *J* 11.7, 10.0 and 4.5, 7-H), 4.29 (2 H, m, 5-H_2), 1.4–2.6 (10 H, m), 1.28, 1.05 and 1.01 (9 H, s, 12-, 13-and 14-H_3) and 1.23 (9 H, s, Bu^t).

Reduction of Sulcatine C **5**.—Sulcatine C **5** (50 mg) was treated with $NaBH_4$ (20 mg) in MeOH (5 cm^3); usual work-up gave a 1:1 mixture of two compounds, which were purified by PLC in hexane–EtOAc (2:1) and identified as sulcatine D **6** (20 mg) and its C-7 epimer **10** (15 mg).

7-epi-*Sulcatine D* **10**. This compound was isolated as a solid, m.p. 145 °C; $[\alpha]_D$ −39.9° (*c* 0.22, MeOH); *m/z* (CI, isobutane) [243 (MH^+), base peak], 225 [(MH^+) − 18] and 207 [(MH^+) − 36]; ^1H and ^{13}C NMR data are reported in Tables 1 and 2.

Sulcatine E **11**.—R_f 0.1, 0.5 (Found: C, 69.9; H, 10.0. $C_{14}H_{24}O_3$ requires C, 69.96; H, 10.07%); *m/z* (CI isobutane) 241 (MH^+) and 223 [(MH^+) − 18, base peak]; ν_{max}(film)/cm^{-1} 3350 (OH) and 1710 (ketone CO); ^1H and ^{13}C NMR data are reported in Tables 1 and 2.

Acetylation of Sulcatine E **11**.—Sulcatine E **11** (20 mg) was acetylated in the usual manner; PLC in hexane–EtOAc (2:1) gave the diacetate derivative **12** (15 mg) as an oil; *m/z* (CI, isobutane) 325 [(MH^+), base peak], 265 [(MH^+) − 60], 205 and 177; ^1H and ^{13}C NMR data are reported in Tables 1 and 2.

Antifungal Tests.—The experiments were performed by means of bioautography; the pure metabolites were loaded in amounts of 5, 10, 50 and 100 μg on a TLC plate, which was, after development, sprayed with a conidial suspension of tested fungi in Czapek Dox Broth. The plate was incubated in a moist chamber and the activity was evidenced by the appearance of a white spot, corresponding to the position of the active metabolite, surrounded by a grey-black fungal growth all over the plate.

Acknowledgements

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